The S100 family of EF-hand calcium-binding proteins: functions and pathology

Beat W. Schäfer and Claus W. Heizmann

Calcium ions as second messengers control many biological processes, at least in part, via interaction with a large number of Ca^{2+}-binding proteins. One class of these proteins shares a common Ca^{2+}-binding motif, the EF-hand. Here, we describe some functional aspects of EF-hand proteins, which have been found recently in different cellular compartments. Novel links between EF-hand proteins, particularly S100 proteins, and specific diseases are now emerging.

THE STIMULATION OF transient increases in intracellular Ca^{2+} controls a wide variety of cellular processes such as cell-cycle progression, differentiation, muscle contraction and enzyme activities. Ca^{2+} overload, as a result of seizures or ischemia, is thought to activate biochemical processes that lead to enzymatic breakdown of proteins and cell death. Furthermore, several disorders, including Alzheimer's disease and neoplastic diseases, are linked to altered Ca^{2+} levels. Intracellular Ca^{2+} levels and Ca^{2+} signalling must therefore be tightly controlled.

Ca^{2+}-coupled responses consist of three major steps (summarized in Fig. 1): (1) activating ligands such as hormones, growth factors, etc. bind to membrane receptors resulting in a rise in the intracellular Ca^{2+} concentration; (2) Ca^{2+} binds to the intracellular mediator proteins such as EF-hand proteins, which transmit the signal by modifying specific target proteins; (3) these altered target proteins coordinate the cellular response to the stimulus. This suggests that the proteins that bind Ca^{2+}, and therefore are involved in the regulation of its concentration, are important in many biochemical processes.

Underlining this importance, the first mutations in two genes coding for EF-hand proteins have recently been described. Inactivation of muscle-specific cysteine protease calpain 3 (which contains four EF hands as Ca^{2+}-binding domains) by mutations result in limb-girdle muscular dystrophy type 2A[1] and a mutation in the calcineurin α gene affects cellular signal transduction pathways in lymphoma cells[2,8]. Undoubtedly, this result will spur on the search for mutations in other EF-hand proteins, which might be candidate genes for different types of disorders.

We describe recent important advances made in our understanding of some novel Ca^{2+}-binding proteins. These proteins are characterized by a common structural motif, the EF hand, after the E- and F-helices of parvalbumin, which binds Ca^{2+} selectively and with high affinity[2,3]. Suggested functions of some members of this family of Ca^{2+}-binding proteins are listed in Table I. Here we will discuss the S100 family of EF-hand proteins, a large and diverse subfamily found extracellularly in the cytoplasm as well as in the nucleus.

B. W. Schäfer and **C. W. Heizmann** are at the Division of Clinical Chemistry, Department of Pediatrics, University of Zurich, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland.

The S100 family of EF-hand proteins

The family of S100 proteins has grown to be one of the largest subfamily of EF-hand proteins. It was originally characterized as a group of abundant low molecular weight (10–12 kDa) acidic proteins that are highly enriched in nervous tissue. They are composed of two distinct EF hands flanked by hydrophobic regions at either terminus and separated by a central hinge region. The carboxy-terminal EF hand is usually referred to as the canonical Ca^{2+}-binding loop and encompasses 12 amino acids, whereas the amino-terminal loop is formed of 14 amino acids and has a lower affinity for Ca^{2+}.

To date, some 17 different proteins have been assigned to the S100 protein family (their main properties are listed in Table II). They show different degrees of homology, ranging from 25% to 65% identity at the amino acid level. Two unusual S100 proteins, profilaggrin and trichohyalin, are actually domains in the preforms of structural proteins synthesized in the epidermis and are cleaved off during maturation[4]. It is not known if they are also stable as independent proteins.

Interestingly, the genes encoding these two proteins were found to be localized in a cluster of at least ten other genes coding for S100 proteins on human chromosome 1q21 (Fig. 2). As several homologues have been localized to a synthenic region on mouse chromosome 3, it is reasonable to postulate that the clustered organization of these S100 genes has been conserved during evolution. The description of the clustered organization of a large number of genes coding for S100 proteins not only raises important questions about the regulation of the individual genes, but also has led to the introduction of a new nomenclature, which is used throughout this review[5]. Whenever the generic term S100 is used, we refer to properties found in most family members.

In the last few years, much information has been obtained on the physical properties of S100 proteins, but only recently have some clues to their functions been gathered, mainly through the identification of novel target proteins.

Functional insights into S100 proteins

The physical and structural properties of S100 proteins suggest that they are trigger or activator proteins, by contrast with other Ca^{2+}-binding proteins that act mainly as buffers. Some family members can form homo- or heterodimers as the functional species, and recently, the structure of a S100A6 dimer has been described (for discussion, see Ref. 6).

A model of the molecular mechanism behind the function of S100 proteins has been adopted from calmodulin, in that the binding of Ca^{2+} to S100 proteins leads to a conformational change that exposes hydrophobic regions in the molecules and allows for target protein interaction. The structural change occurring upon Ca^{2+} binding has been demonstrated for a number of S100 proteins[7]. The affinity of S100 proteins for

 0968-0004/96/$15.00 PII: S0968-0004(96)10020-7

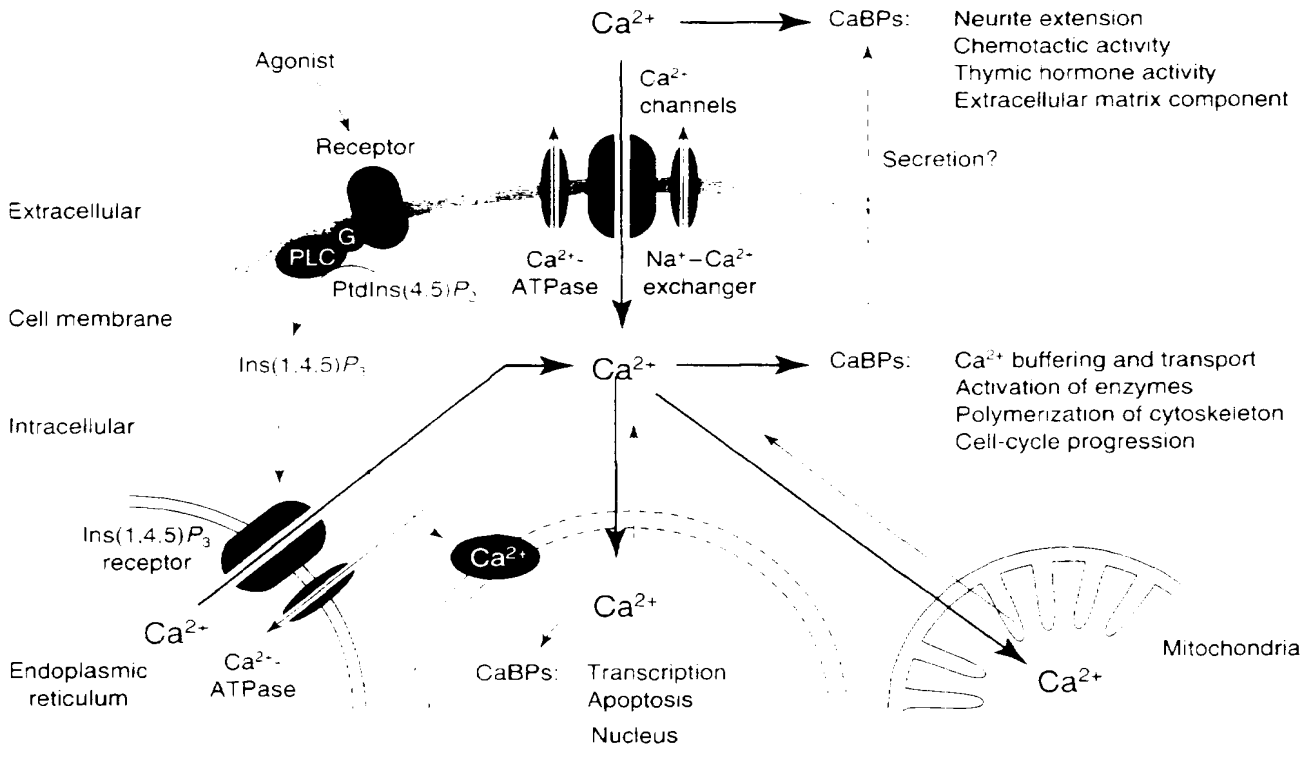

Figure 1

Signal transduction by Ca^{2+}-binding proteins (CaBPs). Influx of Ca^{2+} upon stimulation either from intracellular stores or through different types of Ca^{2+} channels leads to an increase in intracellular Ca^{2+} concentration. This allows CaBPs to bind Ca^{2+}, undergo a conformational change and associate with different target proteins, thereby shaping the biological effects of the Ca^{2+} signal. Ca^{2+}-binding proteins are mainly found in the cytoplasm, but were discovered recently in other compartments of the cell, such as the nucleus or the mitochondria. Abbreviations used: Ins(1,4,5)P_3, inositol 1,4,5-trisphosphate; PLC, phospholipase C; PtdIns(4,5)P_2, phosphatidylinositol 4,5-bisphosphate.

Ca^{2+} is therefore an important parameter, which can be influenced through the binding of Zn^{2+} (increasing the affinity) or K^+ (decreasing the affinity) in several family members. So far, Zn^{2+} binding has been demonstrated directly for S100A6 (Ref. 7), S100B (Ref. 8) and calgranulin C (Ref. 9). Interestingly, S100A3, the S100 protein with the highest cysteine content (10%), binds Zn^{2+} with high affinity, but shows no observable Ca^{2+} binding under physiological conditions[10].

In summary, it can be expected that S100 proteins influence cellular response along the Ca^{2+}-signal-transduction pathway, probably acting at different points in the cascade. Hence, some S100 proteins might initiate the signal transduction cascade, others might modulate the strength of the signal or directly regulate the cellular response, for example, transcription (see Fig. 3).

The signal from the outside

Like several other Ca^{2+}-binding proteins, some S100 proteins (including S100A4, S100A8, S100A9 and S100B) are secreted from different cell types. S100B was demonstrated to stimulate neurite outgrowth, proliferation of melanoma cells and induction of apoptosis in PC12 pheochromocytoma cells (for review, see Ref. 11). Interestingly, nM concentrations of S100B act as a growth and/or differentiation factor, whereas μM concentrations are necessary for induction of apoptosis, indicating that different protein concentrations might be responsible for these diverse effects[12]. Astrocytosis and neurite proliferation also occur in transgenic mice overexpressing S100B, thereby marking one of the few *in vivo* studies available that corroborates *in vitro* experiments[13].

Chemotactic activity on macrophages has been demonstrated for both S100A8 and its murine homolog. In this case, cytokine activity has been reported to occur at even lower concentrations of between 10^{-11}–10^{-13} M. Interestingly, a peptide encompassing the hinge region, located between the two EF hands, has been shown to specifically mediate this effect[14]. For S100A4, no effect of extracellular protein has been demonstrated so far.

The mechanism of action of these diverse functions of S100 proteins is currently not known. However, binding of S100B to glial and neuronal cells at nM concentrations can stimulate Ca^{2+} fluxes[15]. It would, therefore, be an attractive hypothesis to postulate that at least some of the effects seen are mediated by Ca^{2+} as second messenger. Recent evidence supporting this hypothesis was provided by an elegant series of experiments showing that Ca^{2+} transients are both necessary and sufficient to regulate neurite extension[16].

Several questions remain to be addressed regarding the function of extracellular S100 proteins. It is not known how S100 proteins are secreted from cells as they have no classical leader peptide. One possibility is that Ca^{2+} binding results in the exposure of a hydrophobic domain at the amino terminus, allowing an interaction with the

Table I. Suggested functions of some EF-hand Ca^{2+}-binding proteins

Proteins	Functions	Refs
Extracellular		
BM40/SPARC/osteonectin	Extracellular matrix protein; inhibition of cell-cycle progression	a
Nucleobindin	Secreted protein, DNA-binding properties	b, c
β-Parvalbumin (avian thymic hormone)	Thymic hormone activity (lymphocyte maturation)	d
Calmodulin	Development of pre-implantation human embryos	e
Integrin α subunits	Cell adhesion, signal transduction	f
S100 proteins	see Table II	
Intracellular		
Calmodulin	Activator of several enzymes; mediates many Ca^{2+}-dependent processes	g
Troponin C	Modulation of muscle contraction	h
Myosin light chains	Modulation of muscle contraction	i
α-Actinin	Actin-bundling protein	j
α Parvalbumin	Ca^{2+}-buffering and transport; protective role against Ca^{2+} overload	k
Calbindin D_{28k}	Ca^{2+}-buffering and transport; protective role against Ca^{2+} overload	l
Calretinin	Ca^{2+}-buffering and transport; phosphorylation	m
S100 proteins	see Table II	
Recoverin	Phototransduction, activate guanylate cyclase to restore dark state	n
S-modulin	Recoverin homolog	o
Visinin	Phototransduction in cone cells	p
VILIP	Visinin-like protein	q
Neurocalcin/CBP-18	Signal transduction in cortex and cerebellum	r, s
Hippocalcin	Signal transduction in hippocampus	t
Frequenin	Modulation of K^+-channel activity	u
Calcineurin	Calmodulin-dependent phosphatase; target of cyclophilin–cyclosporin complexes	v
Calpain	Ca^{2+}-activated protease	w
Diacylglycerol kinase	Signal transduction	x
Sorcin	Overexpressed in multi-drug resistance	y
Grancalcin	Effector role in neutrophils and monocytes	z
Reticulocalbin	Localized to the endoplasmic reticulum (ER)	aa
ERC-55	Localized to the ER	bb
Calcyphosine	Ubiquitous linkeage of cAMP and Ca^{2+}-phosphatidylinositol pathways	cc
CCBP-23	Regulatory role in signal transduction	dd
R2D5	Regulatory role in olfactory signal transduction	ee
Nuclear		
Calmodulin	Inhibition of transcription; regulation of p68 RNA helicase	ff, gg
Calcineurin	Activation of transcription	hh
Nucleobindin	DNA binding; triggering DNA fragmentation in apoptosis?	ii
NEFA (DNA-binding/EF hand/ acidic amino acid rich)	Domains with two EF-hand motifs; leucine-zipper domain; anti-DNA autoimmunization?	jj
S100 proteins	see Table II	

[a]Sage, E. H. *et al.* (1995) *J. Cell. Biochem.* 57, 127–140; [b]Everitt, E. A. and Sage, E. H. (1992) *Biochem. Cell Biol.* 70, 1368–1379; [c]Miura, K. *et al.* (1994) *Biochem. Biophys. Res Commun.* 199, 1388–1393; [d]Brewer, J. M. *et al.* (1990) *Biochimie* 72, 653–660; [e]Woodward, B. J. *et al.* (1993) *Hum. Reprod.* 8, 272–276; [f]Hogg, N. *et al.* (1995) *Trends Cell Biol.* 4, 379–382; [g]James, P. *et al.* (1995) *Trends Biochem. Sci.* 20, 38–42; [h]Malhotra, A. (1994) *Mol. Cell. Biochem.* 135, 43–50; [i]Allen, B. G. and Walsh, M. P. (1994) *Trends Biochem. Sci.* 19, 362–368; Otto, J. J. (1994) *Curr. Opin. Cell Biol.* 6, 105–109; [k]Heizmann, C. W. and Braun, K. (1992) *Trends Neurosci.* 15, 259–264, and Heizmann, C. W. and Braun, K. (1995); *Calcium-regulation by Calcium-binding Proteins in Neurodegenerative Disorders, Neuroscience Intelligence Unit*, R.G. Landes Company and Springer-Verlag; Chard, P. S. *et al.* (1993) *J. Physiol.* 472, 341–357; [m]Rogers, J. H. (1987) *J. Cell Biol.* 105, 1343–1353; [n]Stryer, L. (1991) *J. Biol. Chem.* 266, 10711–10714; [o]Kawamura, S. (1993) *Nature* 362, 855–857; [p]Polans, A. S. *et al.* (1991) *J. Cell Biol.* 112, 981–989; [q]Lenz, S. E. *et al.* (1992) *Mol. Brain Res.* 15, 133–140; Okazaki, K. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 185, 283–288; [s]Lipp, H. P. *et al.* (1993) *J. Neurochem.* 60, 1639–1649; [t]Kobayashi, M. *et al.* (1992) *Biochem. Biophys. Res. Commun.* 189, 511–517; [u]Pongs, O. *et al.* (1993) *Neuron* 11, 15–28; [v]Mulkey, R. M. *et al.* (1994) *Nature* 369, 486–488; [w]Saido, T. C. *et al.* (1994) *FASEB J.* 8, 814–822; [x]Kai, M. *et al.* (1994) *J. Biol. Chem.* 269, 18492–18498; [y]van der Bliek, A. M. *et al.* (1986) *EMBO J.* 5, 3201–3208; [z]Boyan, A. *et al.* (1992) *J. Biol. Chem.* 267, 2928–2933; [aa]Ozawa, M. and Muramatsu, T. (1993) *J. Biol. Chem.* 268, 699–705; [bb]Chen, J. J. *et al.* (1995) *Science* 269, 529–531; [cc]Lefort, A. *et al.* (1989) *EMBO J.* 8, 111–116; [dd]Sauter, A. *et al.* (1995) *Eur. J. Biochem.* 227, 97–101; [ee]Nemoto, Y. *et al.* (1993) *J. Cell Biol.* 123, 963–976; [ff]Corneliussen, B. *et al.* (1994) *Nature* 368, 760–764; [gg]Buelt, M. K. *et al.* (1994) *J. Biol. Chem.* 269, 29367–29370; [hh]Jain, J. *et al.* (1993) *Nature* 365, 352–355; [ii]Miura, K. *et al.* (1994) *Biochem. Biophys. Res. Commun.* 199, 1388–1393; [jj]Barnikol-Watanabe, S. *et al.* (1994) *Biol. Chem. Hoppe-Seyler* 375, 497–512.

membrane or membranous proteins (e.g. annexins), and thereby resulting in secretion of the molecule. Furthermore, it is unclear if specific cell-surface receptors mediate the effects of S100 proteins. A search for such receptors has so far been unsuccessful.

Modulation of the signal on the inside

The pleiotropic effects reported for intracellular S100 proteins can be divided into several groups. First, S100 proteins can regulate phosphorylation mediated by protein kinase C and thereby can modify the response initiated by the signal transduction cascade[17]. Second, they can regulate the energy metabolism by modulating the activity of several target enzymes, including adenylate cyclase, glyceraldehyde-3-phosphate dehydrogenase and fructose-1,6-bisphosphate aldolase[18]. Third, cell shape can be

Table II. Summary of described properties of members of the S100 protein family

Protein[a]	Cellular localization from main tissues (cells) of synthesis	Metal binding	Target proteins	Suggested functions	Disease association
S100A1 (S100a) (can form homodimers and heterodimers with S100B)	Cytoplasm from neurons, skeletal and heart muscle, kidney	Ca^{2+} ($K_{0.5}$ = 0.02–0.05 mM)[c]	Fructose-1,6-bisphosphate aldolase[d], glycogen phosphorylase[e], adenylate cyclase[f], tubulin[aa] GFAP[bbc], myoD[eee]	Stimulates Ca^{2+}-induced Ca^{2+} release from SR[2], inhibition of microtubule assembly[aa], inhibition of PKC-mediated phosphorylation[i]	Cardiomyopathies[a]
S100A2 (S100L)	Cytoplasm and nucleus from lung and kidney[b]	Ca^{2+}			Breast cancer[j]
S100A3 (S100E)		Ca^{2+}, Zn^{2+} ($K_{0.5}$ = 0.011 mM)[f]			
S100A4 (CAPL)	Extracellular and cytoplasm[c] from fibroblasts, myoepithelial and tumor cells	Ca^{2+} ($K_{0.5}$ = 0.15 mM)[g]	Non-muscle tropomyosin[l], p53[m], non-muscle myosin, heavy chain[n], MAP[o]	Motility and invasion[gg], tubulin polymerization[n]	Metastasis[m,n]
S100A5 (S100D)					
S100A6 (calcyclin) (can form homodimers)	Cytoplasm from fibroblasts and tumor cells	Ca^{2+} ($K_{0.5}$ = 0.32 mM)[h], Zn^{2+} ($K_{0.5}$ = 2 mM)[g]	Annexin II and annexin VI[p], annexin XI[q], glyceraldehyde-3-phosphate dehydrogenase[r]	Stimulation of Ca^{2+}-dependent insulin release[dd], stimulation of prolactin secretion[ee], exocytosis[ff]	Melanoma[n]
S100A7 (psoriasin)	Epithelial cells	Ca^{2+}			Psoriasis[hh]
S100A8 (MRP8) (can form heterodimers with S100A9)	Extracellular and cytoplasm from granulocytes and monocytes	Ca^{2+}	Intermediate filaments (vimentin)[r]	Inhibition of casein kinase[ss], cytokine[hh]	Cystic fibrosis[pp], inflammation[qq]
S100A9 (MRP14)	Extracellular and cytoplasm from granulocytes and monocytes	Ca^{2+}	Intermediate filaments (vimentin)[r]	Inhibition of casein kinase[ss]	Cystic fibrosis[pp], inflammation[qq]
S100A10 (p11)	Cytoplasm from fibroblasts	no	Annexin II[s]	Exocytosis and endocytosis[s]	
S100B (S100b) (can form homodimers and heterodimers with S100A1)	Extracellular and cytoplasm from glial and Schwann cells, melanocytes, chondrocytes and adipocytes	Ca^{2+} ($K_{0.5}$ = 0.02–0.5 mM)[b], Zn^{2+}	Fructose-1,6-bisphosphate aldolase[t], p53[u], calponin[v], tau[v], tubulin[aa], caldesmon[w], neurocalcin[x], neuromodulin (GAP43)[xx], GFAP[aaa], capZ[bbb]	Neurite extension[t] proliferation of melanoma cells[gg], stimulates Ca^{2+} fluxes[y], inhibition of PKC mediated phosphorylation[t], astrocytosis and axonal proliferation[ww], inhibition of microtubule assembly[aa]	Alzheimer's disease[tt], Down's syndrome[ss], tumor marker[ii], amyotrophic lateral sclerosis[vv], epilepsy[ccc]
S100C (can form homodimers)	Smooth and heart muscle	Ca^{2+}	Annexin I[r]		
S100P	Placenta[a]				
Calgranulin C	Cytoplasm of granulocytes	Ca^{2+} Zn^{2+} [z]			
Calbindin 3 (calbindin D_{28k})	Cytoplasm of intestine	Ca^{2+}		Transport function[kk]	
Profilaggrin	Granular cells of epidermis	Ca^{2+} ($K_{0.5}$ = 0.14–1.2 mM)[ll]		Regulation of cell structure	
Trichohyalin	Granular cells of epidermis		Keratin[mm]	Regulation of cell structure	

[a]For the nomenclature, see Schäfer, B. W. *et al.* (1995) *Genomics* 25, 638–643; [b]Glenney, J. R. *et al.* (1989) *J. Cell Biol.* 108, 569–578; [c]Gibbs, F. E. *et al.* (1995) *J. Histochem. Cytochem.* 43, 169–180; [d]Becker, T. *et al.* (1992) *Eur. J. Biochem.* 207, 541–547; [e]Baudier, J. *et al.* (1986) *J. Biol. Chem.* 261, 8192–8203; [f]Fohr, U. G. *et al.* (1995) *J. Biol. Chem.* 270, 21051–21061; [g]Pedrocchi, M. *et al.* (1994) *Biochemistry* 33, 6732–6738; [h]Dell'Angelica, E. C. *et al.* (1994) *J. Biol. Chem.* 269, 28929–28936; Zimmer, D. B. and VanEldik, L. J. (1986) *J. Biol. Chem.* 261, 11424–11428; Zimmer, D. B. and Dubuisson, J. G. (1993) *Cell Calcium* 14, 323–332; [i]Fano, G. *et al.* (1989) *FEBS Lett.* 248, 9–12; Takenaga, K. *et al.* (1994) *J. Cell Biol.* 124, 757–768; [j]Parker, C. *et al.* (1994) *DNA Cell Biology* 13, 343–351; [k]Ford, H. L. and Zain, S. B. (1995) *Oncogene* 10, 1597–1605; [l]Watanabe, Y. *et al.* (1992) *J. Biol. Chem.* 267, 17136–17140; [m]Zeng, F. Y. *et al.* (1993) *Int. J. Biochem.* 25, 1019–1027; [n]Watanabe, M. *et al.* (1993) *Biochem. Biophys. Res. Commun.* 196, 1376–1382; [o]Roth, J. *et al.* (1993) *Blood* 82, 1875–1883; [p]Harder, T. and Gerke, V. (1993) *J. Cell Biol.* 123, 1119–1132; [q]Baudier, J. *et al.* (1992) *Proc. Natl Acad. Sci. USA* 89, 11627–11631; [r]Fujii, T. *et al.* (1994) *Biochem. J.* 116, 121–127; [s]Baudier, J. and Cole, R. D. (1988) *J. Biol. Chem.* 263, 5876–5883; [t]Skriphikova, E. V. and Gusev, N. B. (1989) *FEBS Lett.* 257, 380–382; [u]Okazaki, K. *et al.* (1995) *Biochem. J.* 306, 551–555; [v]Naka, M. *et al.* (1994) *Biochim. Biophys. Acta* 1223, 348–353; [w]Fano, G. *et al.* (1989) *FEBS Lett.* 255, 381–384; [x]Donato, R. (1988) *J. Biol. Chem.* 263, 106–110; [y]Takenaga, K. *et al.* (1994) *Jap. J. Cancer Res.* 85, 831–839; [z]Lakshmi, M. S. *et al.* (1993) *Anticancer Res.* 13, 299–303; [aa]Okazaki, K. *et al.* (1994) *J. Biol. Chem.* 269, 6149–6152; [bb]Murphy, L. C. *et al.* (1988) *J. Biol. Chem.* 263, 2397–2401; [cc]Timmons, P. M. *et al.* (1993) *J. Cell Sci.* 104, 187–196; [dd]Murao, S. *et al.* (1989) *J. Biol. Chem.* 264, 8356–8360; [ee]Lau, W. *et al.* (1995) *J. Clin. Invest.* 95, 1957–1965; [ff]Winningham-Major, F. *et al.* (1989) *J. Cell Biol.* 109, 3063–3071; [gg]Barger, S. W. and VanEldik, L. J. (1992) *J. Biol. Chem.* 267, 9689–9694; [hh]Reeves, R. H. *et al.* (1994) *Proc. Natl Acad. Sci. USA* 91, 5359–5363; [ii]Lee, S. W. *et al.* (1992) *Proc. Natl Acad. Sci. USA* 89, 2504–2508; [jj]Davies, B. R. *et al.* (1993) *Oncogene* 8, 999–1008; [kk]Weterman, M. A. J. *et al.* (1993) *Cancer Res.* 53, 6061–6066; [ll]Hoffmann, H. J. *et al.* (1994) *J. Invest. Dermatol.* 103, 370–375; [mm]Renaud, W. *et al.* (1994) *Biochem. Biophys. Res. Commun.* 201, 1518–1525; [nn]Roth, J. *et al.* (1992) *Immunobiol.* 186, 304–314; [oo]Marshak, D. R. *et al.* (1991) *Neurobiol. Aging* 13, 1–7; [pp]Allore, R. *et al.* (1988) *Science* 239, 1311–1313; [qq]Cochran, A. J. *et al.* (1993) *Melanoma Res.* 3, 325–330; [rr]Alexianu, M. E. *et al.* (1994) *Ann. Neurol.* 36, 846–858; [ss]Presland, R. B. *et al.* (1995) *J. Invest. Dermatol.* 104, 218–223; [tt]Lee, S. C. *et al.* (1993) *J. Biol. Chem.* 268, 12164–12176; [uu]Sheu, F. S. *et al.* (1995) *Arch. Biochem. Biophys.* 316, 335–342; [vv]Christakos, S. *et al.* (1995) *Endocrin. Rev.* 10, 3–26; [ww]Klein, J. R. *et al.* (1993) *Cancer Immunol. Immunother.* 29, 133–138; [xx]Bianchi, R. *et al.* (1994) *Biochim. Biophys. Acta* 1223, 354–360; [yy]Ivanenkov, V. V. *et al.* (1995) *J. Biol. Chem.* 270, 14651–14658; [zz]Pedrocchi, M. *et al.* (1993) *Biochem. Biophys. Res. Commun.* 197, 529–535; [aaa]Griffin, W. S. T. *et al.* (1995) *J. Neurosci.* 65, 228–233; [bbb]Baudier, J. *et al.* (1995) *Biochemistry* 34, 7834–7846.

regulated through influences on the organization of the cytoskeleton. Thus, the polymerization state of all three major types of filaments (microtubules, actin filaments and intermediate filaments) can be influenced by S100 proteins[11]. As most of the interactions have been demonstrated only *in vitro*, the specificity and role *in vivo* is not as clear. Fourth, several members of the S100 family interact with specific annexins, which in turn are targets of several kinases and are, therefore, thought to play an important role in intracellular signal transduction. One S100 protein known not to bind Ca^{2+}, S100A10, forms a tight complex with annexin II, which is thought to be involved in endo- and exocytosis[19].

Several attempts have been made to gain additional insights into the function of S100 proteins through their subcellular localization, but these experiments have proved difficult because antibodies crossreact with several family members. Recently, the generation of novel specific antibodies allowed us to demonstrate that the intracellular

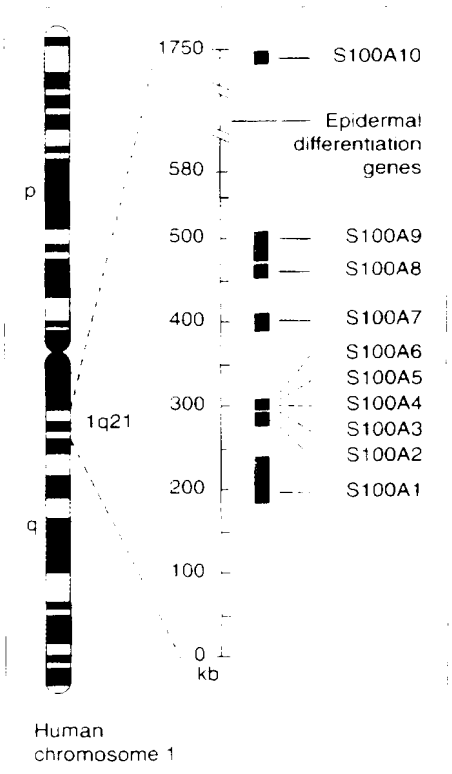

Figure 2

Clustered organization of S100 genes on human chromosome 1q21. A region of 1750 kb is drawn schematically with the genes and their location depicted by open boxes. (For more details, see Ref. 5.) Recently, S100C has also been mapped to chromosome 1q21 (Ref. 29).

distribution of S100 proteins is quite different. This is shown by confocal microscopy of human breast cancer cells labelled for S100A6 (Fig. 4a) and S100A2 (Fig. 4b). S100A6 staining is mostly cytoplasmic, but with a decreasing gradient of staining intensity from the nuclear membrane towards the cell periphery. S100A2 labelling is mostly nuclear, with some granular staining in the cytoplasm. These different intracellular stainings support the view of different functions for S100 proteins (Table II).

S100 or calmodulin?

By contrast to calmodulin, which is ubiquitously expressed, S100 proteins are found in specific cell types, for example neurons, epithelial or glial cells. It is, therefore, of interest to note that an increasing number of common target proteins have been found. Among the proteins regulated both by calmodulin and by one or more members of the S100 family are the cytoskeletal proteins tau, microtubule-associated protein (MAP) and caldesmon, the enzymes adenylate cyclase and glycogen phosphorylase, as well as the cell-cycle-associated proteins p53 and neuromodulin[20] (see Table II).

An interesting example is the recent demonstration that calmodulin and S100A1 are capable of binding to transcription factors of the helix–loop–helix family, thereby modulating the DNA-binding capabilities[21,22]. While in most cases calmodulin and S100 have a similar inhibitory or stimulatory effect on the target protein, there are a few examples of antagonistic effects, for example, tau and glycogen phosphorylase (for review, see Ref. 11). Hence, it might be tempting to postulate a general model for a common function of S100 proteins where they are able to modulate, or sometimes mimic, the effects of the ubiquitous calmodulin in a cell-type-specific manner. However, this hypothesis is clearly too simple and does not explain additional roles of extracellular S100 proteins or the regulation of several specific target molecules such as the annexins.

Association of S100 proteins with human diseases

As discussed above, S100 proteins exert pleiotropic effects in different cell types. Similarly, a wide range of different diseases has been associated with deregulated expression of S100 genes. They can be grouped into two main categories, namely neurological and neoplastic disorders.

Neurological disease. It is worth mentioning that the first link between S100 family members and a specific disease was made for the proteins S100A8 and S100A9, the so-called cystic fibrosis antigens. It was speculated for quite some time that these two S100 proteins could represent the proteins responsible for cystic fibrosis, a speculation now superseded by the cloning of the gene encoding the membrane protein cystic fibrosis conductance regulator (CFTR). Nevertheless, increased coexpression of CFTR, S100A8 and S100A9 could be demonstrated in human cystic fibrosis tracheal-gland cells[23]. Together with the demonstrated role of S100A8 as a potent chemotactic agent[14], it is possible that these S100 proteins might play a role in the chronic inflammation accompanying this disease.

S100B has been linked to several neurological diseases, including Alzheimer's disease, Down's syndrome and recently, epilepsy, because of its synthesis in glial cells and its

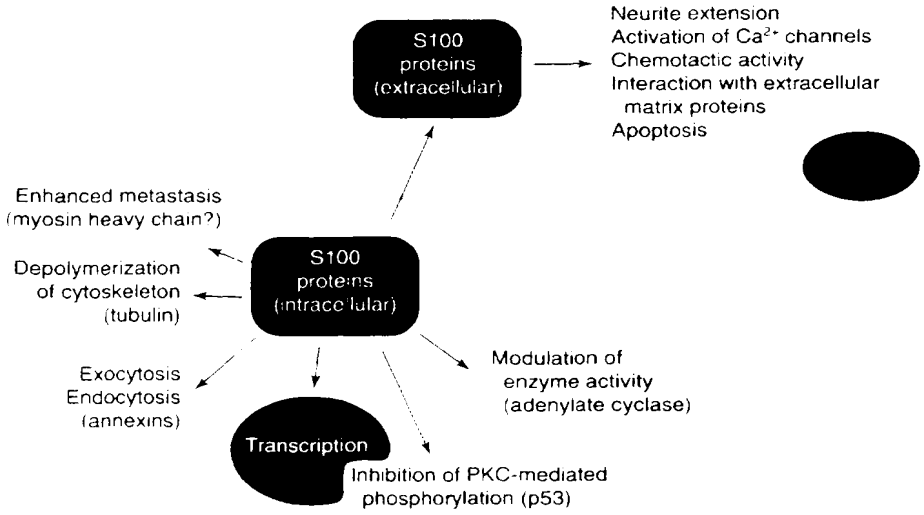

Figure 3

Proposed functions of S100 proteins. S100 proteins might transduce the Ca^{2+} signal producing a variety of biological effects in different cellular compartments as well as acting extracellularly in a paracrine manner. Abbreviation used: PKC, protein kinase C.

localization to human chromosome 21q22. So far, however, only correlative investigations have been carried out. Transgenic mice were generated to examine whether elevated levels of S100B can cause brain dysfunctioning. In mice overexpressing S100B, female hyperactivity and lack of habituation to novelty indicative of hippocampal dysfunction have been observed[24]. It will now be important to extend such studies to animal models of neurological diseases.

Neoplastic disease. Synthesis of S100A2, S100A4, S100A6 and S100B is associated with different neoplastic diseases. S100B, which is upregulated in melanomas, is most widely used as a tumor marker[25]. Its functional role in tumor development, however, has not been investigated. Another link between S100 family members and tumorigenicity comes from the location of the S100 gene cluster, as the chromosome region 1q21 is frequently rearranged in different tumor types, especially in epithelial breast carcinomas[26].

Enhanced synthesis of S100A4 has been demonstrated in metastatic epithelial cells in rodents. In this case, an enhancement of the metastatic behavior of previously low metastatic tumor cells was achieved through ectopic expression of the gene encoding S100A4 and demonstrating a causal role of S100A4 in this process[27]. The mechanism of action, however, is currently unclear. Several different mechanisms have been suggested, among which is the binding of S100A4 to either myosin heavy chain (influencing cell morphology and motility) or p53 (inactivation of tumor suppressor function). Another possibility is that extracellular S100A4 influences cell behavior. It will be necessary to use additional overexpression experiments in different cell types from different species to solve this issue.

Interestingly, and contrary to S100A4, the production of S100A2 was found to be downregulated in tumorigenic cells compared with normal breast epithelial cells. One speculation, therefore, might be that S100A2 and S100A4 influence cell behavior in a similar way, although in opposite directions. In this case, too, ectopic expression experiments in cell culture and animal models are needed to prove or disprove this hypothesis.

As described above, most evidence linking EF-hand Ca^{2+}-binding proteins (in particular S100 proteins) with disease states is only circumstantial. Clearly, providing functional evidence will be a major focus of research on Ca^{2+}-binding proteins in the future, and the establishment of animal models will be of critical importance. Also, mutations affecting either synthesis or structure might be found in association with specific diseases and could help to further improve our understanding of the diverse functions of the EF-hand Ca^{2+}-binding protein family.

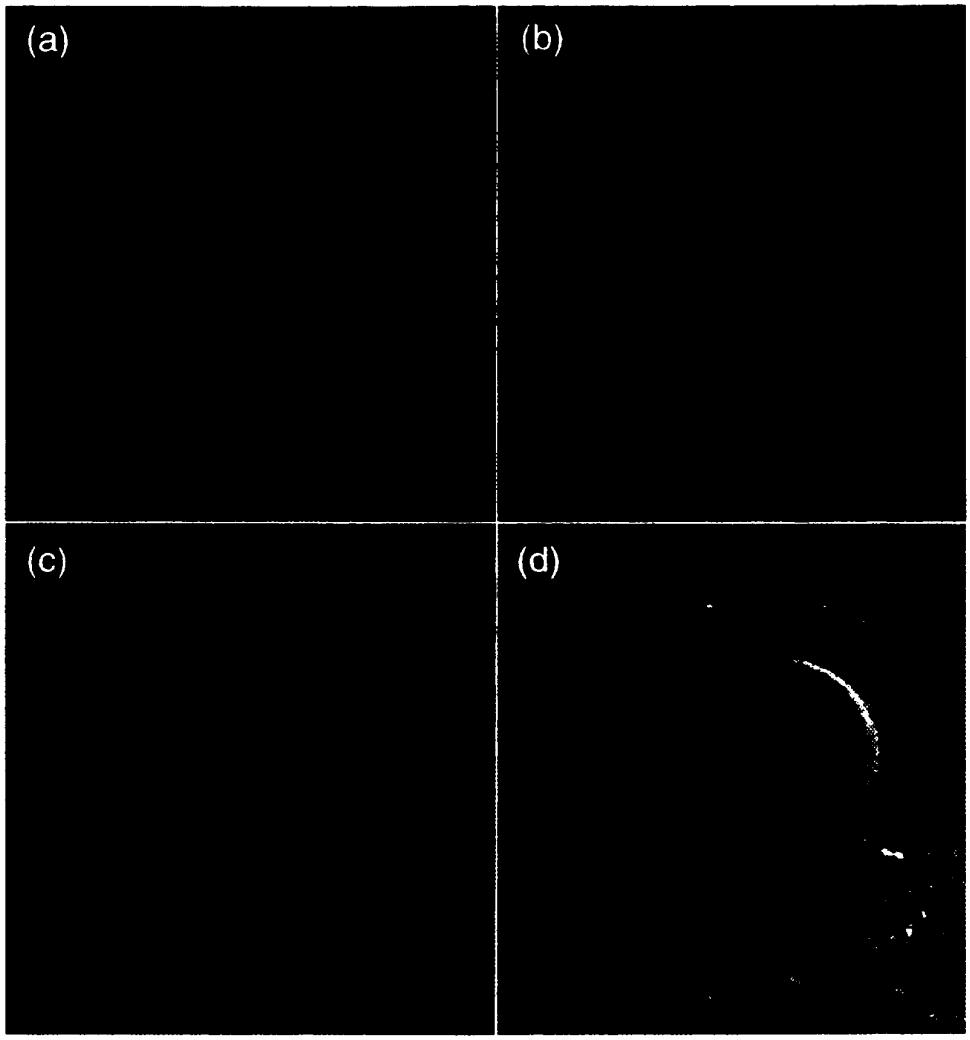

Figure 4

Double immunofluorescence labelling for **(a)** S100A6 and **(b)** S100A2 in human breast cancer cells (HBL-100). Confocal microscopy demonstrates different intracellular staining patterns: **(a)** S100A6, mostly cytoplasmic; **(b)** S100A2, mostly nuclear; **(c)** superimposed double staining; and **(d)** phase contrast picture. Bar = 10 μm (courtesy of T. Bächi, Laboratory for Electron Microscopy and E. Ilg, Department of Pediatrics, Division of Clinical Chemistry, University of Zurich).

Acknowledgements

We would like to thank A. Rowlerson, A. Lauber, B. Thöny and M. Killen for critical reading of this manuscript. This study was supported in part by the Wilhelm Sander Stiftung (FRG) and the Swiss National Science Foundation.

References

1 Richard, I. *et al.* (1995) *Cell* 81, 27–40
2 Kretsinger, R. H. (1980) *CRC Crit. Rev. Biochem.* 8, 119–174
3 Heizmann, C. W. and Hunziker, W. (1991) *Trends Biochem. Sci.* 16, 98–103
4 Presland, R. B., Bassuk, J. A., Kimball, J. R. and Dale, B. A. (1995) *J. Invest. Dermatol.* 104, 218–223
5 Schäfer, B. W. *et al.* (1995) *Genomics* 25, 638–643
6 Potts, B. C. M. *et al.* (1995) *Nat. Struct. Biol.* 2, 790–797

7 Pedrocchi, M. *et al.* (1994) *Biochemistry* 33, 6732–6738.
8 Baudier, J., Glasser, N. and Gérard, D. (1986) *J. Biol. Chem.* 261, 8192–8203
9 Dell'Angelica, E. C., Schleicher, C. H. and Santome, J. A. (1994) *J. Biol. Chem.* 269, 28929–28936
10 Fohr, U. G. *et al.* (1995) *J. Biol. Chem.* 270, 21056–21061
11 Zimmer, D. B., Cornwall, E. H., Landar, A. and Song, W. (1995) *Brain Res. Bull.* 37, 417–429
12 Fano, G. *et al.* (1995) *Prog. Neurobiol.* 46, 71–82
13 Reeves, R. H. *et al.* (1994) *Proc. Natl Acad. Sci. USA* 91, 5359–5363
14 Lau, W., Devery, J. M. and Geczy, C. L. (1995) *J. Clin. Invest.* 95, 1957–1965
15 Barger, S. W. and VanEldik, L. J. (1992) *J. Biol. Chem.* 267, 9689–9694
16 Gu, X. and Spitzer, N. C. (1995) *Nature* 375, 784–787
17 Baudier, J. *et al.* (1992) *Proc. Natl Acad. Sci. USA* 89, 11627–11631
18 Donato, R. (1991) *Cell Calcium* 12, 713–726
19 Harder, T. and Gerke, V. (1993) *J. Cell Biol.* 123, 1119–1132
20 James, P., Vorherr, T. and Carafoli, E. (1995) *Trends Biochem. Sci.* 20, 38–42
21 Baudier, J. *et al.* (1995) *Biochemistry* 34, 7834–7846
22 Cornelussen, B. *et al.* (1994) *Nature* 368, 760–764
23 Renaud, W., Merten, M. and Figarella, C. (1994) *Biochem. Biophys. Res. Commun.* 201, 1518–1525
24 Gerlai, R. and Roder, J. (1995) *J. Psychiatry Neurosci.* 20, 105–112
25 Cochran, A. J. *et al.* (1993) *Melanoma Res.* 3, 325–330
26 Engelkamp, D. *et al.* (1993) *Proc. Natl Acad. Sci. USA* 90, 6547–6551
27 Davies, B. R. *et al.* (1993) *Oncogene* 8, 999–1008
28 Fruman, D. A. *et al.* (1995) *Mol. Cell. Biol.* 15, 3857–3863
29 Hoog-Lutz, C. *et al.* (1995) *Int. J. Cancer* 63, 297–303

The biology of HMG-CoA reductase: the pros of contra-regulation

Randolph Hampton, Dago Dimster-Denk and Jasper Rine

Hydroxymethylglutaryl-CoA reductase (HMG-R) is a key enzyme in the mevalonate pathway, from which thousands of molecules are derived including cholesterol and prenyl moieties. The regulation of HMG-R is complex and includes feedback control, cross-regulation by independent biochemical processes and contra-regulation of separate isozymes. From studies in yeast, these separate modes of regulation can be considered in an integrated fashion.

HMG-CoA REDUCTASE (HMG-R), by catalyzing the synthesis of mevalonic acid from HMG-CoA, plays a critical role in the production of the large family of molecules derived from mevalonic acid by the mevalonate pathway (Fig. 1). Although the mevalonate pathway is best known as the source of sterols such as cholesterol[1], products of the mevalonate pathway comprise a staggering array of molecules that vary among species, cell type and physiological state[2–4]. *Saccharomyces cerevisiae* has been used as a model for HMG-R synthesis and has revealed numerous perplexing features of HMG-R structure and regulation. In this review, we will discuss the HMG-R protein family, including the structural themes and the regulatory modes encountered in diverse organisms. We will then focus on HMG-R in yeast, where most of the features of HMG-R biology are found and where they can be viewed in an integrated fashion.

R. Hampton is at the Department of Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA; **D. Dimster-Denk** is at the Division of Biology, HHMI, California Institute of Technology, Pasadena, CA 91125, USA; and **J. Rine** is at the Division of Genetics, MCB Dept, 401 Barker Hall, University of California at Berkeley, Berkeley, CA 94720, USA.

The HMG-R protein family

HMG-R is present in archaebacteria[5] and presumably in all eukaryotes[6–10]. A more distant relative of HMG-R has also been described in eubacteria[11]. The members of the HMG-R protein family consist of an extremely conserved catalytic domain and a highly diversified membrane-anchoring region (Fig. 2). All versions of the protein have a highly conserved carboxy-terminal domain that is responsible for the conversion of HMG-CoA to mevalonic acid. Examples of the extent of conservation of catalytic function include the human enzyme functioning in yeast[12] and the archaeal enzyme being potently inhibited by lovastatin, a clinically used competitive inhibitor of mammalian HMG-R[5].

The individual catalytic domains of HMG-R are fused to a bewildering array of amino-terminal regions (Fig. 2). In most cases these amino-terminal regions appear to contain membrane-spanning sequences that anchor the protein in the endoplasmic reticulum (ER), but the archaeal enzyme is fused to a hydrophilic 21-residue peptide[5]. The primary sequence and the resultant structures of these amino termini are quite different. Those in plants have two putative transmembrane spans[2,10], the metazoan HMG-Rs all have eight transmembrane spans[6,7,13], whereas yeast HMG-R appears to have seven transmembrane spans[12,14]. Furthermore, although comparisons among members of the same phylogenetic kingdom (e.g. tomato vs potato, or hamster vs sea urchin) often reveal primary sequence similarity in the amino-terminal regions, comparisons between the amino termini of different kingdoms reveal no similarity at all. Given that the catalytic domain can function without membrane attachment, both in the natural setting of the archaeal HMG-R[5] and in engineered forms of other HMG-R proteins[15,16], why do so many HMG-Rs appear to be attached to a membrane?

The HMG-R family also displays another level of complexity. In many instances, including all plants examined[4,17], *S. cerevisiae*[12] and *Dictyostelium* (A. De Lozonne, pers. commun.), there are

 0968-0004/96/$15.00 PII: S0968-0004(96)10017-7